presented in the application (see pages 29-31). In addition, attention is directed to the attached manuscript in which the inventors report the inhibition of HIV infection of mononuclear phagocytes by anti-CD44 antibodies and hyluronate. Further, attention is directed to the attached abstract published in Clinical Research (41(2):322A (1993)) in which the inventors report the differential modulation of HIV infection of a T cell line by expression of transfected CD44 isoforms. The data presented in the abstract underscore the importance CD44 expression to HIV infection.

The Examiner's comments to the contrary, the *in vitro* data provided in the application can be expected to be predictive of efficacy *in vivo*. The Examiner has not indicated why such would not be the case. Numerous reports have demonstrated a strong correlation between the ability of HIV to infect cells *in vitro* (determined by cell syncitia formation, viral reverse transcriptase production and viral p24 production) and the ability to cause infection *in vivo*. Likewise, protection against infection *in vivo* with an agent (for example, a vaccine or antibodies) correlates with *in vitro* assays. (See, for example, Haynes, Science 260:1279 (1993); Girard et al, Proc. Natl. Acad. Sci. USA 88:542 (1991); and Berman et al, Nature 345:622 (1990) (copies to follow)). Further, the Examiner is reminded that the recited agents can be used to lessen infectability of HIV in

products designed for infusion (eg blood derived transfusion products). Thus, the method in no way has as its sole utility "a potential role as an object of use testing".

In view of the above, the Examiner is urged to reconsider the rejection based on lack of utility and withdraw same.

This application is submitted to be in condition for allowance and a Notice to that effect is respectfully requested.

Respectfully submitted,

NIXON & VANDERHYE P.C.

By Mary J. Wilson
Mary J. Wilson
Reg. No. 32,955

MJW:tat

1100 North Glebe Road
8th Floor
Arlington, Virginia 22201-4714
Telephone: (703) 816-4000
Facsimile: (703) 816-4100

Full title:

Inhibition of HIV-1-BaL Infection of Mononuclear Phagocytes by Anti-CD44 Antibodies*

by

Emilia D. Rivadeneira†, Barton F. Haynes‡,§ , and J. Brice Weinberg‡,‖

* Supported in part by the VA Medical Research Service, NIH grant AR39162, the James Swiger Hematology Research Fund, and NIH Training Grant 5-T32-AI07392.

Departments of Pediatrics†, Medicine‡,

Microbiology–Immunology§, and Obstetrics-Gynecology‖

VA and Duke University Medical Centers

Durham, North Carolina 27705

Abstract

Cellular CD4 is the primary membrane molecule that binds HIV-1 through interaction with viral gp120. Membrane glycolipids and cell adhesion molecules have also been noted to be involved in the interaction of HIV-1 with cells and in syncytia formation in infected cells. The purpose of this study was to determine the role of the cell adhesion molecule CD44 in HIV-1 infection of cells. Both normal blood monocytes and lymphocytes expressed CD44 as determined by flow cytometry using the anti-CD44 antibody A3D8. Anti-CD44 monoclonal antibodies A3D8 and A1G3 inhibited infection of monocytes and peritoneal macrophages with HIV-1-BaL, but had no effect on HIV-1-IIIB infection of mitogen-stimulated lymphocytes, or cells of the T lymphocyte line . CD44 monoclonal antibodies were not toxic for monocytes, and the observed inhibitory effect of CD44 monoclonal antibodies was not dependent on complement. Hyaluronic acid, one ligand of CD44, also inhibited HIV-1-BaL infection of monocytes. These results suggest that CD44 may be a determinant of HIV-1 infection of mononuclear phagocytes *in vitro*.

Introduction

Cellular CD4 has been recognized as the predominant membrane protein that interacts with HIV-1. In most cells, CD4 serves as the receptor that binds HIV-1 gp120 initiating events that lead to cell-viral membrane fusion and internalization of HIV into the susceptible cell (1-3). The ability of HIV to infect some CD4 negative cells (4-6), and the observation that gp120-CD4 interactions may not be the sole determinants of HIV-1 infectivity and spread (7) have prompted the search for additional cell surface molecules involved in HIV-1 infection . For example, although the lymphocyte function associated-type 1 (LFA-1) molecule has been shown not to be an important determinant of HIV-1 infection of cells, it is required for efficient fusion of HIV-1-infected cells and syncytia formation (8-12). Furthermore, in CD4 negative neural cell lines and colon epithelial cells, investigators have found that the membrane lipid galactosyl ceramide can serve as a receptor for HIV-1, and bind gp120 (5, 6). Also, investigators have noted that even though certain viral strains preferentially infect either monocytes or lymphocytes (monocytotropic or lymphocytotropic viral strains), both strains interact with CD4 (13, 14). This implies that other cellular molecules may play a role in determining tropism.

To determine the role of non-CD4 cell membrane molecules in HIV-1 infection of monocytes and lymphocytes, we have been studying various anti-cell adhesion molecule antibodies in *in vitro* assays of HIV-1 infection. We report here that antibodies against CD44 effectively inhibit the infection of human mononuclear phagocytes (monocytes and peritoneal macrophages) with the monocytotropic HIV-1 strain BaL. However, the antibodies have no effect on the infection of normal blood lymphocytes and a T lymphoid cell line with the lymphotropic HIV-1 strain IIIB. These studies indicate that CD44 may be an important determinant of HIV-1 infection of mononuclear phagocytes.

Methods and materials

Cells: Human monocytes, peritoneal macrophages, lymphocytes, and blood mononuclear cells were isolated by sequential ficoll-Hypaque and Percoll gradient centrifugations as noted before

(15-17), under a protocol approved by the Duke University and VA Medical Centers Institutional Review Boards. Monocytes and peritoneal macrophages, after isolation, adherence, and washing were greater than 95% pure, as determined by Wright's and nonspecific esterase stains. Mononuclear phagocytes were cultured with Dulbecco's modified Eagle medium of low endotoxin content and 10% human serum. The continuous human T lymphoid cell line CEM is maintained in our laboratory and is mycoplasma free. Normal blood lymphocytes activated by phytohemagglutinin and interleukin-2 were prepared as noted before (17). Lymphocyte cultures were done in RPMI-1640 medium of low endotoxin content with 10% heat inactivated fetal bovine serum. Cell surface antigen analysis by indirect immunofluorescence using a FACSTAR analyzer was done as described before (18).

Viral strains: HIV-1-BaL (monocytotropic) and HIV-1-IIIB (lymphocytropic) viral strains were used (19, 20). Cells were infected at a multiplicity of infection of approximately 0.01 to 0.1.

Monoclonal antibodies: Murine monoclonal antibodies directed against CD44 [A3D8 and A1G3 (21, 22)], and MHC class I (3F10) were used as ascites or as IgG purified using a Staph protein A column. Monoclonal antibodies A3D8 and A1G3 recognize different epitopes of the CD44 molecule (21, 22). Ascites from mice bearing the parent mouse myeloma cell line was used as control (P3) in some experiments. Antibodies were added to the cells at initiation of the cultures, and left in throughout the culture period. Cells were inoculated with virus within 24 hours of initiation of the experiments. Supernatant media were collected every 3 to 7 days, and terminated 18 to 21 days after inoculation.

Assessment of viral infection: Morphologic cytopathic effects were determined by observation of live cells with inverted phase contrast microscopy, and of methanol-fixed cells stained with Wright's stain at the end of the experiments. Reverse transcriptase was measured after 14 days of culture as noted before determining counts by scintillation counting or by the use of a phosphoimager (17, 23). Reverse transcriptase results from a typical experiment using

monocytes and HIV-1-BaL revealed a mean of 50125 cpm for monocytes with HIV-1-BaL, while those for lymphocytes using HIV-1-IIIB were 90518 cpm.

Other methods and reagents: All other reagents were from Sigma Chemical Company (St. Louis, MO). The presence of endotoxin was determined by the limulus amebocyte lysate colorimetric assay (Whittaker Laboratories, Bar Harbor, ME).

Results

CD44 is expressed by numerous cell types, including erythrocytes, lymphocytes, and mononuclear phagocytes (24). As seen in figure 1, the anti-CD44 antibody A3D8 reacted strongly with normal blood lymphocytes and monocytes isolated by density gradient sedimentation as noted before (15) (figure 1). Monocytes had a slightly greater density of CD44 than did lymphocytes.

As noted before by us and others, HIV-1-BaL infection of human monocytes and peritoneal macrophages resulted in cytopathology (multinucleated giant cells), and in the production of supernatant viral RT and p24. If the cultures were done in the presence of antibodies against CD44, there was a consistent decrease in the HIV-1-induced cytopathology (figure 2) and of RT levels in culture supernatant medium (figure 3). The antibodies against CD44 inhibited HIV-1 infection of monocytes, while an antibody against MHC class I antigen (3F10) did not appreciably alter the RT levels (figure 3). The inhibition was seen when using ascites or purified A3D8 IgG (figure 3).

Investigators have noted that as mononuclear phagocytes differentiate [either *in vitro* or *in vivo* (23, 25)], they become more susceptible to productive infection with HIV-1. We found that freshly isolated peritoneal macrophages [the *in vivo* differentiated form of blood monocytes (23)], like monocytes, were inhibited in their ability to be infected with HIV-1-BaL by monoclonal antibodies against CD44 (figure 3). To determine the possible role of complement in the inhibition, we cultured monocytes in serum which had been heated to inactivate complement.

Under these culture conditions, the anti-CD44 antibodies inhibited HIV-1-BaL productive infection to a comparable degree (75 to 80% inhibition). As opposed to the inhibition of HIV-1 infection of mononuclear phagocytes with anti-CD44 antibodies, these antibodies had no inhibitory effect on HIV-1-IIIB infection of proliferating blood lymphocytes (figure 3). Likewise, the anti-CD44 antibody A3D8 did not inhibit HIV-1-IIIB infection of cells of the T lymphocyte cell line CEM. For example, eight days after inoculation with undiluted HIV-1-IIIB, supernatants from control CEM cells had RT values of 332,758 cpm (100%), while those with A3D8 (1:100 dilution) were 373,896 (112%).

Certain isoforms of CD44 are known to bind hyaluronan (hyaluronic acid) and to serve as the cellular receptor for this extracellular matrix component (24, 26). To determine if soluble hyaluronan could inhibit infection of monocytes, we incubated varying amounts of hyaluronan with monocytes and determined the ability of HIV-1-BaL to infect these cells. Hyaluronan inhibited HIV-1-BaL infection of monocytes, with an ID_{50} of approximately 5 ug/ml. Other extracellular matrix components had no or little inhibitory effect (figure 4). However, hyaluronan did not alter HIV-1-IIIB infection of normal peripheral blood lymphocytes or CEM cells (data not shown).

Discussion

In this study, we have demonstrated that productive infection of human monocytes with HIV-1-BaL (as manifest by cytopathology and production of viral RT) can be inhibited by antibodies against the membrane glycoprotein CD44 and by the CD44 ligand hyaluronan. However, infection of CD44 positive, mitogen-activated T lymphocytes or CEM cells with the lymphotropic virus HIV-1-IIIB was not inhibited by the CD44 monoclonal antibodies or by hyaluronan.

Human CD44 (previously known as Pgp-1, HCAM, Hermes antigen, and the lymphocyte homing receptor) is now known to be a family of related glycoproteins of different function formed apparently by alternative splicing of RNA (24, 27-30). These different isoforms may

mediate various functions including (*i*) serving as the membrane receptor for hyaluronan; (*ii*) anchoring cells to the extracellular matrix by binding hyaluronan, fibronectin, or collagen; (*iii*) binding to the cytoskeletal protein ankyrin; (*iv*) mediating leukocyte binding to endothelial cells, and leukocyte aggregation; (*v*) serving as a leukocyte receptor involved in lymphocyte co-mitogenesis and monocyte monokine secretion; and (*vi*) in determining metastatic behavior of certain tumor cells (24). Soluble CD44 has been described in tissue fluids and plasma (31), and soluble CD44 or CD44 in liposomes can apparently interfere with the normal function of CD44 *in vitro* or *in vivo* (31-33). An intact CD44 cytoplasmic domain is critical for the functional activity of CD44 (26); interaction with protein kinase C may be important in the cell signaling pathways (34, 35).

Gallatin and co-workers noted earlier that in macaques infected with simian immunodeficiency virus (SIV), there was a selective depletion of CD4 positive-CD44 ("heterotypic adhesion receptor") "high" cells (36). Furthermore, the $CD4^{+}$,$CD44^{hi}$ cells were much more susceptible to productive SIV infection *in vitro* (36). This suggests that CD44 might be a determinant of lentivirus infection of cells *in vivo*. Our experiments demonstrate that monocyte CD44 could be involved in the *in vitro* HIV-1 infection of human monocytes. The mechanism(s) by which anti-CD44 antibodies inhibit this infection is not known. It is possible that monocyte CD44 serves as an auxiliary binding molecule for HIV-1 membrane components (e.g., gp120). Alternately, engaging CD44 with either antibody or ligand (hyaluronan) could cause "activation" of monocytes to express other anti-viral mechanisms [e.g., expression of interferon-alpha (37, 38)]. Expression of different CD44 isoforms in various cell types (e.g., monocytes and lymphocytes) could determine cellular responses to anti-CD44 antibodies and hyaluronan, and explain the differences we note in this inhibition of HIV-1 infection of lymphoid cells and monocytes. Our findings suggest that *cellular* CD44 (in addition to cellular CD4 and viral gp120) may be an important determinant of viral tropism.

The full elucidation of the mechanism(s) underlying the observed inhibition may aid in the understanding of HIV-1 infection. This work may help in the development of novel strategies for preventing or controlling HIV-1 infection; anti-CD44 antibodies, CD44 ligands, or soluble CD44 could prove useful therapeutically.

Acknowledgments

We thank Dr. Tom Matthews for helpful discussions, and A.M.F. Streiland, C. McDanal, T.S. Wortham, and R. Scearce for technical assistance.

Figure legends

Figure 1—Flow cytometric analysis of blood lymphocytes and monocytes using with anti-CD14 and anti-CD44 monoclonal antibodies. Monocytes and lymphocytes were separated by sequential centrifugation as described in the Methods, and analyzed for expression for CD14 with antibody LeuM3 (panel A) and CD44 with antibody A3D8 (panel B). The solid line is for monocytes, and the dotted line is for lymphocytes.

Figure 2—Photomicrograph of control and HIV-1-BaL-inoculated monocytes without or with monoclonal anti-CD44 antibody A3D8. Monocytes were placed into microtiter wells ± antibody ± HIV-1-BaL, and cultured for 21 days. The anti-CD44 monoclonal antibody A3D8 prevents the cytopathic effect caused by HIV-1-BaL.

A, control monocytes; **B**, control monocytes inoculated with HIV-1-BaL; **C**, A3D8-treated control monocytes; **D**, A3D8-treated monocytes inoculated with HIV-1-BaL. (Wright's stain; original magnification 40X).

Non-inoculated monocytes (A and C) are generally mononuclear; while those inoculated with HIV-1-BaL and cultured without antibody A3D8 contain numerous multinucleated giant cells (syncytia) with 4 to 100 nuclei (B). HIV-1-BaL-inoculated monocytes cultured with antibody A3D8 have no multinucleated giant cells (syncytia) (D).

Figure 3—Inhibition of HIV-1-BaL productive infection of blood monocytes and peritoneal macrophages (but not blood lymphocytes) by anti-CD44 monoclonal antibodies. The data is expressed as percent of control for the reverse transcriptase values measured 14 days after inoculation.

● = control;

▲ = monocytes with unheated human serum;

◆ = peritoneal macrophages with unheated human serum;

○ = monocytes with heat-inactivated human serum;

× = monocytes with purified A3D8 antibody;

▶ = peripheral blood lymphocytes

→ = mean

Figure 4—Inhibition of HIV-1-BaL productive infection of blood monocytes by hyaluronic acid. Monocytes were seeded with various doses of hyaluronic acid or chrondroitin sulfate (SO_4), and inoculated with HIV-1-BaL. Supernatant reverse transcriptase was measured after 14 days of cultures. This shows results of a representative experiment expressed as percent of control for the reverse transcriptase values.

References

1. **Fisher, R. A., J. M. Bertonis, W. Meier, V. A. Johnson, D. S. Costopoulos, T. Liu, R. Tizard, B. D. Walker, M. S. Hirsch, R. T. Schooley, R. A. Flavell.** 1988. HIV infection is blocked in vitro by recombinant soluble CD4. *Nature 331:76.*

2. **Dalgleish, A. G., P. C. L. Beverley, P. R. Clapham, D. H. Crawford, M. F. Greaves, R. A. Weiss.** 1984. The CD4 (T4) antigen is an essential component of the receptor for the AIDS retrovirus. *Nature 312:763.*

3. **Sattentau, Q. J., R. A. Weiss.** 1988. The CD4 antigen: physiological ligand and HIV receptor. *Cell 52:631.*

4. **Tateno, M., F. Gonzalez-Scarano, J. A. Levy.** 1989. Human immunodeficiency virus can infect CD4-negative human fibroblastoid cells. *Proc Natl Acad Sci USA 86:4287.*

5. **Harouse, J. M., M. A. Laughlin, C. Pletcher, H. M. Friedman, F. Gonzalez-Scarano.** 1991. Entry of Human Immunodeficiency Virus-1 into Glial Cells Proceeds via an Alternate, Efficient Pathway. *J Leuk Biol 49:605.*

6. **Harouse, J. M., S. Bhat, S. L. Spitalnik, M. Laughlin, S. Stefano, D. H. Silberberg, F. Gonzalez-Scarano.** 1991. Inhibition of entry of HIV-1 in neural cell lines by antibodies against galactosyl ceramide. *Science 253:320.*

7. **Maddon, P. J., A. G. Dalgleish, J. S. McDougal, P. R. Clapham, R. A. Weiss.** 1986. The T4 gene encodes the AIDS virus receptor and is expressed in the immune system and the brain. *Cell 47:333.*

8. **Hansen, J.-E. S., C. Nielsen, L. R. Mathiesen, J. O. Nielsen.** 1991. Involvement of lymphocyte function-associated antigen-1 (LFA-1) in HIV infection: inhibition by monoclonal antibody. *Scand J Inf Dis 23:31.*

9. **Hildreth, J. E. K., R. J. Orentas.** 1989. Involvement of a leukocyte adhesion receptor (LFA-1) in HIV-induced syncytium formation. *Science 244:1075.*

10. **Pantaleo, G., G. Poli, L. Butini, C. Fox, A. I. Dayton, A. S. Fauci.** 1991. Dissociation Between Syncytia Formation and HIV Spreading - Suppression of Syncytia Formation Does Not Necessarily Reflect Inhibition of HIV Infection. *Eur J Immunol 21:1771.*

11. **Pantaleo, G., L. Butini, C. Graziosi, G. Poli, S. M. Schnittman, J. J. Greenhouse, J. I. Gallin, A. S. Fauci.** 1991. Human immunodeficiency virus (HIV) infection in CD4+ T lymphocytes genetically deficient in LFA-1: LFA-1 is required for HIV-mediated cell fusion but not for viral transmission. *J Exp Med 173:511.*

12. **Valentin, A., K. Lundin, M. Patarroyo, B. Asjo.** 1990. The leukocyte adhesion glycoprotein CD18 participates in HIV-1-induced syncytia formation in monocytoid and T cells. *J Immunol 144:934.*

13. **Hwang, S. S., T. J. Boyle, H. K. Lyerley, B. R. Cullen.** 1992. Identification of envelope V3 loop as the major determinant fo CD4 neutralization sensitivity of HIV-1. *Science 257:535.*

14. **O'Brien, W. A., I. S. Y. Chen, D. D. Ho, E. S. Daar.** 1992. Mapping genetic determinants for human immunodeficiency virus type 1 resistance to soluble CD4. *J Virol 66:3125.*

15. **Weinberg, J. B., J. J. Muscato, J. E. Niedel.** 1981. Monocyte chemotactic peptide receptor. Functional characteristics and ligand-induced regulation. *J Clin Invest 68:621.*

16. **Weinberg, J. B., A. F. Haney.** 1983. Spontaneous tumor cell killing by human blood monocytes and human peritoneal macrophages: lack of alteration by endotoxin or quenchers of reactive oxygen species. *J Natl Cancer Inst 70:1005.*

17. **Weinberg, J. B., T. J. Matthews, B. R. Cullen, M. H. Malim.** 1991. Integration of HIV-1 genomic DNA into chromosomal DNA of human monocytes does not require cellular DNA synthesis. *J Exp Med 174:1477.*

18. **Magrinat, G., S. N. Mason, P. S. Shami, J. B. Weinberg.** 1992. Nitric oxide modulation of human leukemia cell differentiation and gene expression. *Blood 80:1880.*

19. **Gartner, S., P. Markovits, D. M. Markovitz, M. H. Kaplan, R. C. Gallo, M. Popovic.** 1986. The role of mononuclear phagocytes in HTLV-III/LAV infection. *Science 233:215.*

20. **Hwang, S. S., T. J. Boyle, H. K. Lyerley, B. R. Cullen.** 1992. Identification of the envelope V3 loop as the primary determinant of cell tropism in HIV-1. *Science 253:71.*

21. **Telen, M. J., G. S. Eisenbarth, B. F. Haynes.** 1983. Human erythrocyte antigens: regulation of expression of a novel red cell surface antigen by the inhibitor Lutheran In(Lu) gene. *J Clin Invest 71:1878.*

22. **Haynes, B. F., E. A. Harden, M. J. Telen, M. E. Hemler, J. L. Strominger, T. J. Palker, R. M. Scearce, G. S. Eisenbarth.** 1983. Differentiation of human T lymphocytes. I. Acquisition of a novel human cell surface protein (p80) during normal intrathymic T cell maturation. *J Immunol 131:1195.*

23. **Shadduck, P. P., J. B. Weinberg, A. F. Haney, J. A. Bartlett, A. J. Langlois, D. P. Bolognesi, T. J. Matthews.** 1991. Lack of enhancing effect of human anti-human immunodeficiency type 1 (HIV-1) antibody on HIV-1 infection of human blood monocytes and peritoneal macrophages. *J Virol 65:4309.*

24. **Haynes, B. F., H. X. Liao, K. L. Patton.** 1991. The transmembrane hyaluronate receptor (CD44): multiple functions, multiple forms. *Cancer Cells 3:347.*

25. **Olafsson, K., M. S. Smith, P. Marshburn, S. G. Carter, S. Haskill.** 1991. Variation of HIV infectibility of macrophages as a function of donor, stage of differentiation, and site of origin. *J AIDS 4:154.*

26. **Lesley, J., Q. He, K. Miyake, A. Hamann, R. Hyman, P. W. Kincade.** 1992. Requirements for hyaluronic acid binding by CD44: a role for the cytoplasmic domain and activation by antibody. *J Exp Med 175:257.*

27. **Lesley, J., R. Hyman.** 1992. CD44 can be activated to function as a hyaluronic acid receptor in normal murine T cells. *Eur J Immunol 22:2719.*

28. **Goldstein, L. A., D. F. H. Zhou, L. J. Picker, C. N. Minty, R. F. Bargatze, J. F. Ding, E. C. Butcher**. 1989. A human lymphocyte homing receptor, the Hermes antigen, is related to cartilage proteoglycan core and link proteins. *Cell 56:1963*.

29. **Stamenkovic, I., A. Aruffo, M. Amiot, B. Seed**. 1991. The hematopoietic and epithelial forms of CD44 are distinct polypeptides with different adhesion potentials for hyaluronate-bearing cells. *EMBO J 10:343*.

30. **Screaton, G. R., V. B. Martyn, D. G. Jackson, F. B. Cornelis, U. Gerth, J. I. Bell**. 1992. Genomic structure of DNA encoding the lymphocyte homing receptor CD44 reveals at least 12 alternatively spliced exons. *Proc Natl Acad Sci USA 89:12160*.

31. **Haynes, B. F., L. P. Hale, K. L. Patton, M. E. Martin, R. M. McCallum**. 1991. Measurement of an adhesion molecule as an indicator of inflammatory disease activity. Up-regulation of the receptor for hyaluronate (CD44) in rheumatoid arthritis. *Arthritis Rheum 34:1434*.

32. **Watson, S. R., C. Fennie, L. A. Lasky**. 1991. Neutrophil influx into an inflammatory site inhibited by a soluble homing receptor-IgG chimaera. *Nature 349:164*.

33. **Sy, M.-S., Y.-J. Guo, I. Stamenkovic**. 1992. Inhibition of tumor growth in vivo with a soluble CD44-immunoglobulin fusion protein. *J Exp Med 176:623*.

34. **Bourguignon, L. Y. W., E. L. Kalomiris, V. B. Lokeshwar**. 1991. Acylation of the lymphoma transmembrane glycoprotein GP85 may be required for GP85-ankyrin interaction. *J Biol Chem 266:11761*.

35. **Bourguignon, L. Y., V. B. Lokeshwar, J. He, X. Chen, G. J. Bourguignon**. 1992. A CD44-like endothelial cell transmembrane glycoprotein (GP116) interacts with extracellular matrix and ankyrin. *Mol Cell Biol 12:4464*.

36. **Gallatin, W. M., M. J. Gale, P. A. Hoffman, D. M. Willerford, K. E. Draves, R. E. Beneveniste, W. R. Morton, E. A. Clark**. 1989. Selective replication of simian immunodeficiency virus in a subset of CD4+ lymphocytes. *Proc Natl Acad Sci USA 86:3301*.

37. **Gendelman, H. E., L. M. Baca, C. A. Kubrak, P. Genis, S. Burrous, R. M. Friedman, D. Jacobs, M. S. Meltzer**. 1992. Induction of IFN-alpha in Peripheral Blood Mononuclear Cells by HIV-Infected Monocytes - Restricted Antiviral Activity of the HIV-Induced IFN. *J Immunol 148:422*.

38. **Gendelman, H. E., R. M. Friedman, S. Joe, L. M. Baca, J. A. Turpin, G. Dveksler, M. S. Meltzer, C. Dieffenbach**. 1990. A selective defect of interferon α production in human immunodeficiency virus-infected monocytes. *J Exp Med 172:1433*.

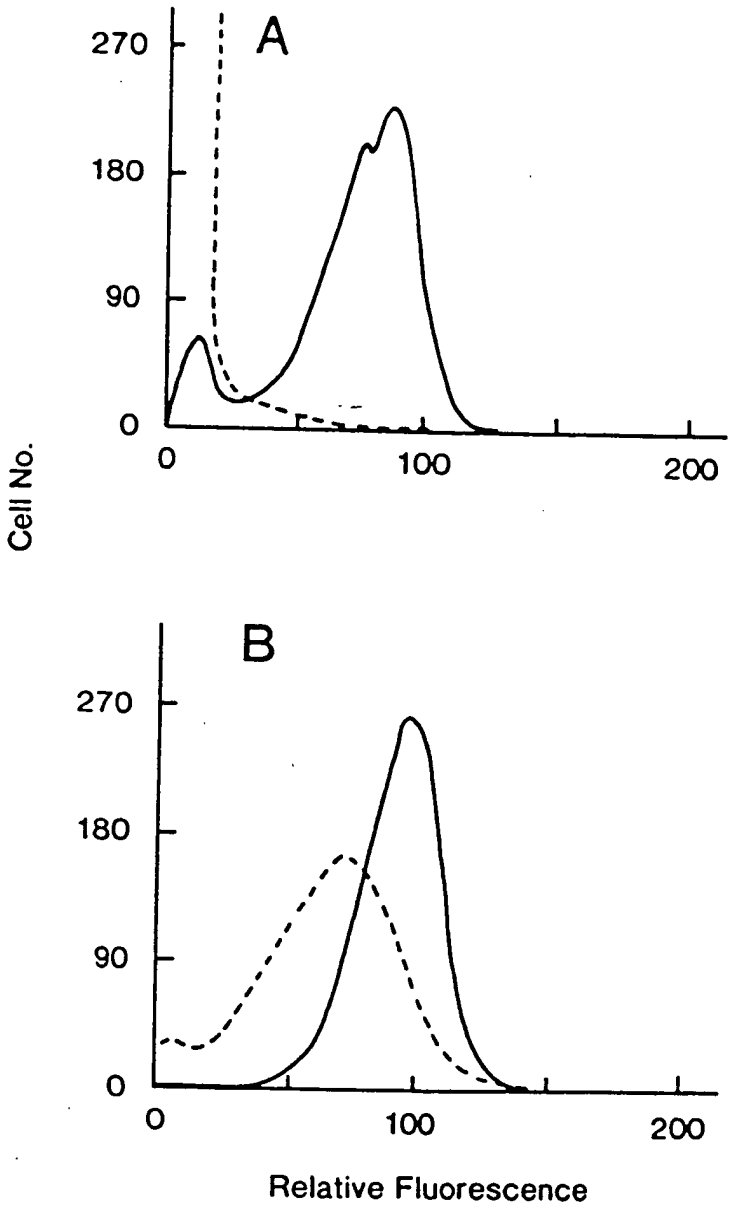

Fig 1 - Fluorescence of cells

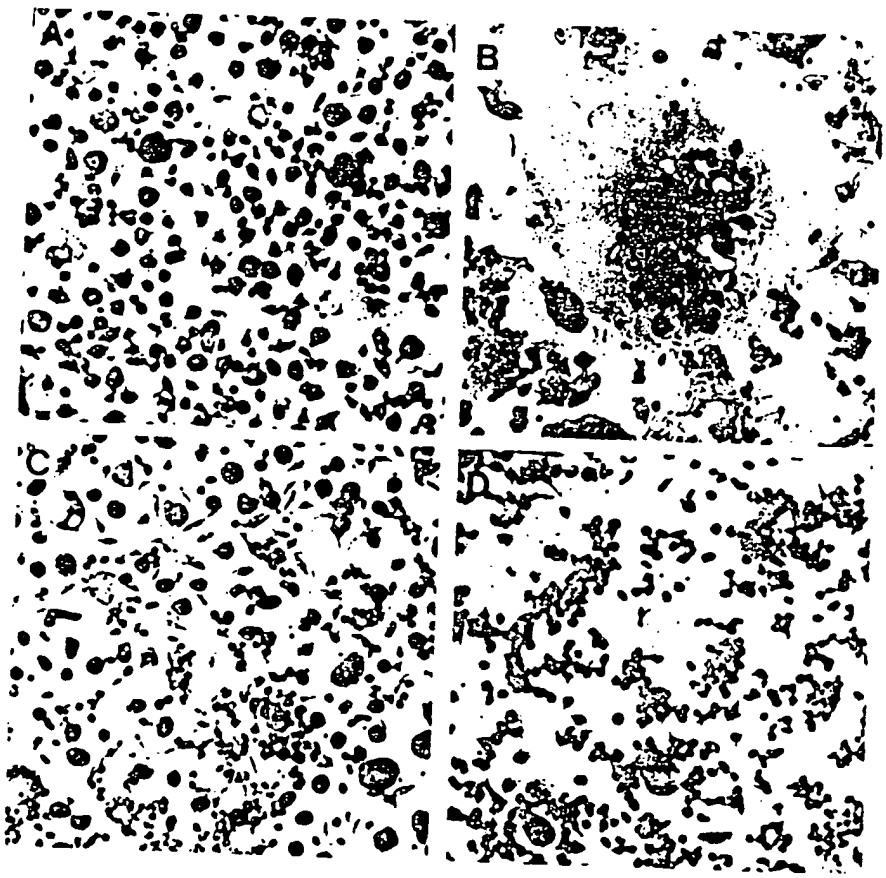

Fig 2

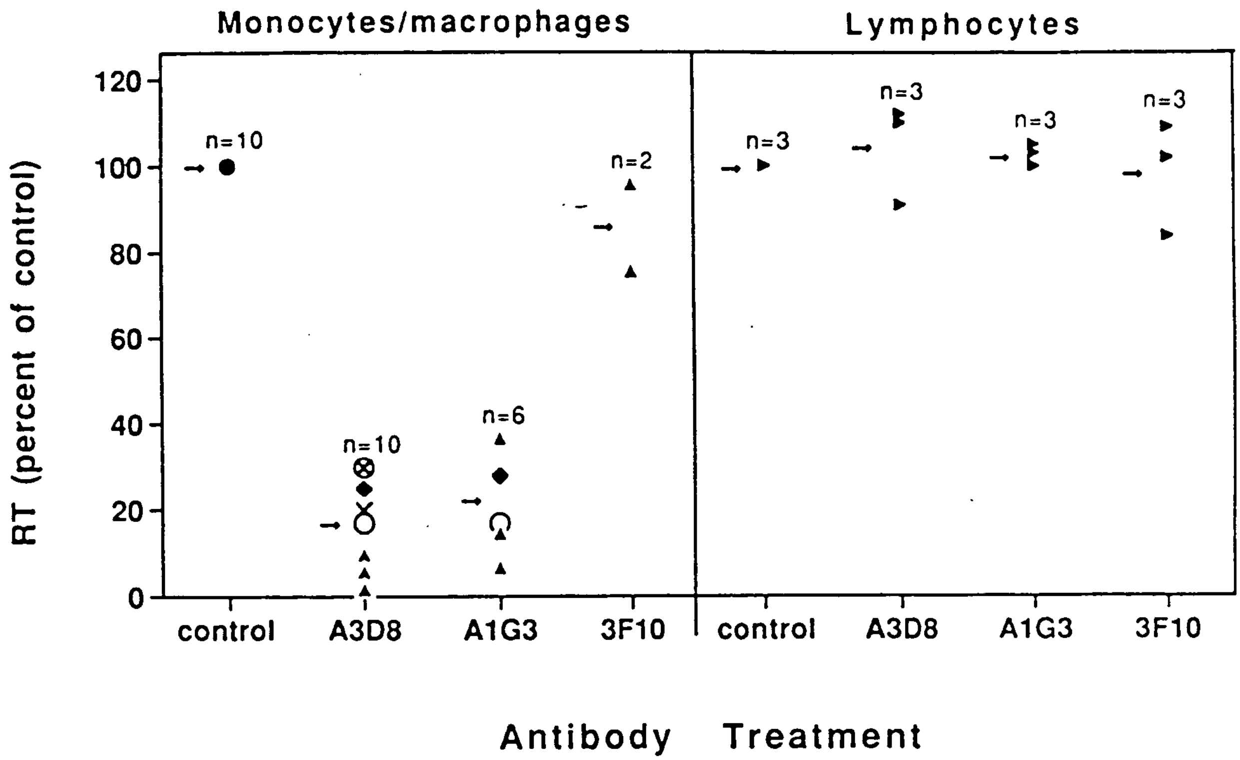
Monocytes/macrophages
Lymphocytes
RT (percent of control)
120
100
80
60
40
20
0
n=10
n=2
n=6
n=10
n=3
n=3
n=3
n=3
control
A3D8
A1G3
3F10
control
A3D8
A1G3
3F10
Antibody Treatment

Fig 3

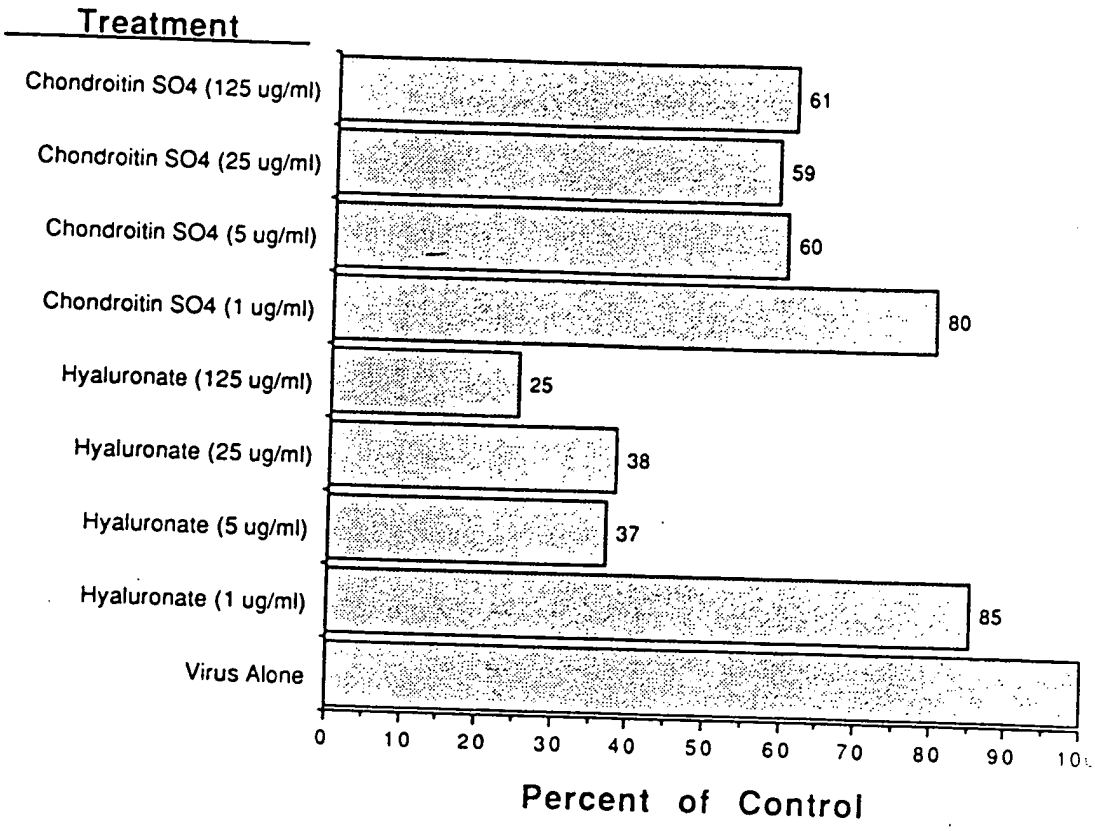
Treatment
Chondroitin SO4 (125 ug/ml)
Chondroitin SO4 (25 ug/ml)
Chondroitin SO4 (5 ug/ml)
Chondroitin SO4 (1 ug/ml)
Hyaluronate (125 ug/ml)
Hyaluronate (25 ug/ml)
Hyaluronate (5 ug/ml)
Hyaluronate (1 ug/ml)
Virus Alone
61
59
60
80
25
38
37
85
0 10 20 30 40 50 60 70 80 90 10
Percent of Control

Fig 9

LIPOPHOSPHOGLYCAN FROM LEISHMANIA DONOVANI DOWN-REGULATES IL-1β GENE EXPRESSION IN THP-1 CELLS. DH Hatziegeorgiou, Y Zhang*, S Turco*, WN Rom and JL Ho. Div. of International Medicine, Cornell University Medical College, New York, NY, Div. of Pulmonary Critical Care Medicine, New York University Medical Center, New York, NY and Department of Biochemistry, University of Kentucky, Chandler Medical Center, Lexington, KY.

Human infection by *Leishmania donovani* (L.d.) causes visceral leishmaniasis which is associated with an immune defect, characterized by T-cell unresponsiveness to L.d. antigens and diminished production of IL-1β by peripheral blood mononuclear cells (J.L. Ho et al. *J Infect Dis 1992;165:1094*). IL-1β produced by antigen presenting cells and monocytes/macrophages plays an important role in T-cell activation and proliferation. Previous studies have shown that human monocytes infected by L.d. and stimulated by LPS or *S. aureus*, have diminished total amount of IL-1β, TNF-α and c-*fos* mRNA. Lipophosphoglycan (LPG), the most abundant molecule on *Leishmania*, has been shown to decrease LPS induction of IL-1β synthesis by human monocytes. To further explore the level of IL-1β gene regulation by L.d. components, we measured by densitometry the mRNA levels extracted from a human monocytic cell line THP-1, after Northern hybridization on nylon membrane and probing with ^{32}P-labeled IL-1β. When cells where pretreated with 10-15 μg/mL LPG (from L.d.) for 2.5 hours and further stimulated by 1-3 μg/mL LPS (from *E. coli*) for 4 hours, a 30-40% suppression of mRNA levels was observed as compared to LPS treatment alone. To examine the mechanism for the suppression of IL-1β gene expression we used an isolated IL-1β genomic DNA (-1110/+290) from a human placenta genomic library (Y. Zhang, M. Yu and W.N. Rom, *Clin Res 1991;39:289A*). A series of 5' flanking region deletion mutants were created and linked to chloramphenicol acetyl-transferase (CAT) reporter gene. THP-1 cells were transiently transfected by these plasmid constructs and after pretreatment by LPG and stimulation by LPS, the activation of IL-1β promoter was determined by measuring the CAT activity from the cell extracts. A 40% suppression of CAT activity was found in cells pretreated with 10 μg/mL LPG and stimulated by 3 μg/mL LPS. The major suppressive element in response to LPG seems to be located within -310/+290 of IL-1β gene, while deletion to -57 resulted in loss of LPG suppressive effect. These data suggest that LPG from L.d. suppresses IL-1β expression by acting at 5' elements located on -310 to +290 region of the gene.

MECHANISM AND TRANSCRIPTIONAL EFFECTS OF HIV-1 SUPERINFECTION OF CHRONICALLY INFECTED CELL LINES. IH Kim, MT Vahey*, RJ McLinden*, JD Mosca*, DS Burke*, and RR Redfield*. Walter Reed Army Institute of Research and the Henry M. Jackson Foundation, Rockville, Maryland.

Acute infection of T-cell lines by HIV-1 is associated with downregulation of the CD4 receptor and resistance to further viral infection. A number of cell lines, such as ACH2, are chronically infected with HIV-1 and serve as models for chronic infection. Using the polymerase chain reaction to discriminate between HIV_{LAI} and HIV_{RF}, we show that ACH2 cells (HIV_{LAI}) can be superinfected and that the frequency of superinfection increases with time. Superinfection of a HIV_{IIIB}-chronically infected H9 line was also achieved. Filtered supernatant from an ACH2/HIV_{RF} superinfection transmits both HIV_{LAI} and HIV_{RF}, suggesting productive infection. Antibody against CD4 blocks superinfection, implying superinfection occurs through a gp120/CD4 interaction. Reverse transcription of ACH2 mRNA from 14 and 28 days post-infection allowed analysis of RF- and LAI-specific transcripts. Superinfection of ACH2 with HIV_{RF} results in an increase in mRNA of both strains. With time there is an apparent increase in the relative expression of HIV_{RF} compared with HIV_{LAI}. Interestingly, by day 28, when only 0.5% of the ACH2 cells contained superinfecting HIV_{RF}, roughly 50% of the HIV-specific full-length mRNA was HIV_{RF}. Sequencing of PCR-derived LTR fragments from A3.01 cells acutely infected with HIV_{RF} or ACH2 cells shows differences in the critical U3-R region. Functional differences in CAT activity are also observed. Establishment of an ACH2 cell line infected with both host HIV_{LAI} and superinfecting HIV_{IIIB} permitted study of the rate of induction of full-length HIV transcripts. Transcription from the superinfecting strain was more rapidly induced by PMA. These data establish the feasibility of superinfection and offer insight into the effect of superinfection on the expression of host and superinfecting strain mRNA and raise the possiblity that HIV-based retroviral vectors might be used to target HIV-infected cells and to modify viral expression within those cells.

DIFFERENTIAL MODULATION OF HIV-1 INFECTION OF A T CELL LINE BY EXPRESSION OF TRANSFECTED CD44E AND CD44H ISOFORMS. ED Rivadeneira,* H-X Liao,* DL Sauls,* BF Haynes, and JB Weinberg. VA and Duke University Medical Centers, Durham, NC.

HIV-1 infection of susceptible cells is largely dependent on binding of viral gp120 to cellular CD4. In earlier work, however, we noted that antibodies against the transmembrane protein CD44 inhibited infection of mononuclear phagocytes. Different isoforms of CD44 appear to be important in hyaluronate binding, in leukocyte homing, in establishing tumor cell metastases, and in modulation of lymphocyte and monocyte function. The purpose of this study was to determine the effects of transfection of the CD4 positive-CD44 negative Jurkat T cell line with cDNA for CD44E or CD44H, two different isoforms of CD44. Cells transfected with CD44E displayed high levels of cell surface CD44E with less surface CD4 than Jurkat-parent cells, and grew to similar densities. Although the Jurkat-parent cells and Jurkat-CD44E transfectants could not be infected by the monocytotropic virus strain HIV-1$_{BaL}$, both cell lines were successfully infected by the lymphocytotropic virus strain HIV-1$_{IIIB}$. Jurkat-CD44E cells were more susceptible to infection with HIV-1$_{IIIB}$, as compared to the Jurkat-parent cells. They had earlier syncytia formation, and had higher levels of viral reverse transcriptase. At 9 days after inoculation, the ability of HIV-1$_{IIIB}$ to infect the Jurkat-CD44E cells was approximately 9 times greater than that for the Jurkat-parent cells as determined by $TCID_{50}$. In contrast to Jurkat-parent and Jurkat-CD44E cells, Jurkat-CD44H cells were much less susceptible to HIV-1 infection displaying no evidence of infection at day 9, despite expressing high levels of CD4. All three cell lines bound gp120 equally well, suggesting that CD44 modulation of HIV-1 infection-expression was independent of gp120-CD4 binding. Expression of different cellular CD44 isoforms appears to be an important determinant of HIV-1 infection and expression *in vitro*.

AMINOGLYCOSIDE RESISTANCE DUE TO Tn*4001* AMONG GRAM-POSITIVE COCCAL BLOOD ISOLATES. MM Huycke, KS Gilmore*, MS Gilmore*, T Verville*, University of Oklahoma Health Sciences Center, Oklahoma City, OK.

High-level resistance to gentamicin (MIC ≥ 2 g/L; HLGR) and other aminoglycosides among *Enterococcus faecalis* (Ef), *Staphylococcus aureus* (Sa), and coagulase-negative staphylococcal (Cns) strains is often mediated by a 6'-aminoglycoside acetyl-transferase-2"-aminoglycoside phosphotransferase. The gene for this bifunctional enzyme (*aac(6')-aph(2")*) resides on Tn*4001* (and several other transposons), and is flanked by the inverted repeat IS*256*. We screened epidemiologically distinct Ef (n = 28), Sa (n = 36), and Cns (n = 19) blood isolates by PCR using internal primers to *aac(6')-aph(2")* to determine the frequency of HLGR due to this gene. Each of 13 Ef, 6 Sa, and 13 Cns HLGR strains had a PCR-amplified DNA product of predicted size (985 basepairs). Each strain also hybridized by dot blot to a digoxigenin-labeled internal fragment of *aac(6')-aph(2")*. One Ef and Sa strain susceptible to gentamicin were also positive by PCR and dot blot for *aac(6')-aph(2")*. Strains containing *aac(6')-aph(2")* were subjected to additional PCR using an IS*256* primer and one or the other *aac(6')-aph(2")* primers. Each Sa (n = 7) and Cns (n = 13) strains had amplified DNA products for the left and right ends of Tn*4001* (1511 and 1919 basepairs, respectively). For Ef (n = 14), the left end of Tn*4001* was found in 4, left and right ends in 9, and neither end in 1 strain. Using these primers, PCR amplification of a *Escherichia coli* lysate containing pIP800, derived from a HLGR Ef strain described in 1980, produced the 985 basepair fragment of *aac(6')-aph(2")* and the left end of Tn*4001*. By Southern blot, the DNA products amplified using IS*256* and *aac(6')-aph(2")* primer combinations hybridized to the internal fragment of *aac(6')-aph(2")*. High-level aminoglycoside resistance due to *aac(6')-aph(2")* often resides on Tn*4001* (or closely related genetic elements), and may explain in part the recent worldwide emergence of this phenotype among human Gram-positive coccal pathogens.

ACTIVATION-DEPENDENT TRANSLOCATION OF $p22^{rac1}$ AND $p22^{CDC42Hs}$ FROM NEUTROPHIL CYTOSOL TO PLASMA MEMBRANE. MR Philips*, AS Feoktistov*, MH Pillinger* and G Weissmann, Dept. of Med., NYU Med.Ctr., New York, NY.

Ras-related GTP-binding proteins are targeted to membranes by prenylation and carboxyl methylation of their C-termini. Among the cytosolic components of the neutrophil NADPH oxidase assembled at the plasma membrane (PM) of activated cells is a geranylgeranylated member of the *rho* family of *ras*-related proteins, $p22^{rac2}$. We have shown that GTPγS stimulates both carboxyl methylation and translocation from cytosol (CS) to PM of neutrophil $p22^{rac2}$ in a reconstituted cell-free system (*Science* in press). We now show that carboxyl methylation is not required for translocation. Subcellular localization of *ras*-related proteins in resting neutrophils revealed that >98% of the immundetectable $p22^{rac2}$ was cytosolic whereas the *ras* family protein $p21^{rap1}$ was >98% membrane-associated. A 2.8±1.3 (n=3) fold increase in the PM/CS ratio of $p22^{rac2}$ was observed in neutrophils stimulated with FMLP (100 nM, 1min) prior to cavitation. When PM and CS were mixed (1:4, w:w) and subsequently separated into soluble and particulate fractions, 9±1% (n=4) of $p22^{rac2}$ sedimented with PM. In the presense of GTPγS (100 μM) the membrane-associated fraction increased to 44±6% ($p<0.01$). The translocation of $p22^{rac2}$ did not appear to depend on methylation since neither the methyl donor, *S*-adenosylmethionine, nor its competitive inhibitor, *S*-adenosylhomocysteine, affected GTPγS-mediated translocation. Moreover, neither *N*-acetyl-*S*-farnesylcysteine nor *N*-acetyl-*S*-geranylgeranylcysteine, inhibitors of prenylcysteine-specific carboxyl methyltransferase, diminished the translocation of $p22^{rac2}$. Another *rho* protein, $p22^{CDC42Hs}$, was also translocated from CS to PM in a GTPγS-dependent fashion whereas the membrane-associated fraction of $p21^{rap1}$ was unaffected by GTPγS. These data suggest that chemoattractants induce translocation of cytosolic $p22^{rac2}$ and $p22^{CDC42Hs}$ by stimulating GDP/GTP exchange and that translocation does not require carboxyl methylation.

EVIDENCE FOR TISSUE-SPECIFIC REV ACTIVITY IN MICE TRANSGENIC FOR HIV GENES. L.A. Bruggeman,* P.J. Nelson,*+ J.B. Kopp, R.C. Gallo,+ A.L. Notkins,** P.E. Klotman and M.E. Klotman+ Laboratory of Developmental Biology and Laboratory of Oral Medicine, National Institute of Dental Research and +Laboratory of Tumor Cell Biology, National Cancer Institute, Bethesda, MD.

The HIV-1 genome codes for at least nine known proteins that are expressed from unspliced as well as alternatively spliced mRNAs. The transregulatory protein, Rev, enhances the expresssion of unspliced genomic RNA as well as singly spliced mRNAs coding for structural proteins. The protein binds to the Rev responsive element (RRE) within these viral messages, however, the precise mechanism by which this leads to enhanced expression is not known. To further understand this regulation, we utilized a transgenic mouse model containing a subgenomic, non-infectious HIV-1 provirus. We characterized viral mRNA in different tissues by Northern analysis and reverse transcriptase/polymerase chain reaction (RT/PCR) amplification of viral messages. RT/PCR analysis of the multiply spliced messages demonstrated a complex pattern of alternatively spliced viral messages similar to what has been previously described. Northern analysis revealed that the level of expression of the transgene varied in each tissue. Furthermore, the relative abundance of the 2 kb messages compared to singly spliced 4 kb and unspliced 7 kb RNA varied in different tissues. Two patterns of expression were observed. In skin, tail and muscle there was a predominance of the multiply spliced 2 kb mRNA, a pattern consistent with low Rev activity. In contrast, the thymus and kidney had almost equivalent expression of 2 kb, 4 kb and 7 kb messages consistent with efficient Rev activity.

Therefore, in HIV-1 transgenic mice, there is a tissue-specific pattern of Rev activity suggesting the role for tissue-specific factors in modulating or substituting for Rev activity.